

Brief Research

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Update on Urinary Tract Infections in Dogs in a Tropical Island and Antimicrobial Susceptibility of *Escherichia Coli* Isolates for the Period 2010-2016

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ABSTRACT

Background: Published information on bacterial urinary tract infection (UTI) in dogs in tropical countries, including the Caribbean is scanty. Monitoring antimicrobial resistance helps in empirical selection of drugs. The objectives of this study were to examine the diagnostic laboratory records on canine UTI in Grenada for the period of 2009 to 2016, and to identify drugs which are most likely to be effective.

Methods: Urine samples from 468 dogs suspected of urinary tract infection (UTI) were quantitatively cultured, and the bacterial isolates were identified by phenotypic methods. Antimicrobial susceptibility tests were done using the standard disk diffusion method against 6 or more drugs.

Results: Of the 150 culture-positive samples, 71.3% were positive for Gram-negative bacterial UTI, and *Escherichia coli* (*E. coli*) were the predominant species. Antimicrobial resistance was least to enrofloxacin (7.8%) and most to tetracycline (58.7%) when both Gram-negative and Gram-positive isolates were considered together. With regard to *E. coli*, none of the 39 isolates tested were resistant to imipenem, a last-resort drug, but 42% isolates showed resistance to 2 or more classes of drugs, including 2 isolates with multi-resistance to 6 classes of drugs. One *E. coli* isolate was resistant to 5 classes of drugs, including resistance to extended spectrum cephalosporins (ESC), as indicated by resistance to cefotaxime and 4 other cephalosporins.

Conclusions: This study confirms that *E. coli* is the common bacterial species associated with UTI in dogs in Grenada, and that resistance is minimal to enrofloxacin when all bacteria are considered together. However, emerging *E. coli* with multi-drug resistance to ESC is of concern, and continued monitoring is important for prudent use of antimicrobial drugs, and to detect emerging trends in veterinary medicine, including those of public health significance such as resistance to ESC, which may be transferred to human pathogens.

KEYWORDS: Urinary tract infection; Dogs; Grenada; Bacteria; *Escherichia coli*; Antibiotic Susceptibility.

ABBREVIATIONS: UTI: Urinary Tract Infection; ESC: Extended Spectrum Cephalosporins; *E. coli*: *Escherichia coli*.

INTRODUCTION

Urinary tract infections (UTI) are the most common cause of urinary tract disease in dogs. Approximately 14% of all dogs will acquire bacterial UTI during their lifetimes.¹ The most frequently isolated bacterium from dogs with UTI is *Escherichia coli* (*E. coli*), followed by *Staphylococcus* spp., *Proteus* spp., *Streptococcus* spp., *Klebsiella* spp., *Enterobacter* spp., and

Pseudomonas spp.^{2,3} Suggested drugs for treatment of UTI include amoxicillin-clavulanate, trimethoprim-sulfa, cephalexin, fluoroquinolones, tetracycline, and ampicillin. Alternative drugs include gentamicin and chloramphenicol.^{3,4} Fluoroquinolones such as enrofloxacin are particularly effective in treating UTI due to the high drug concentrations achievable within the urinary tract.⁵ In a study in the U.S, over 80% of urinary tract isolates were found to be susceptible to enrofloxacin.⁵

Despite several recent publications on bacterial UTI in dogs from Europe and the United States,⁶⁻⁹ there is scanty information from tropical areas. The results of a study on the canine urine samples from cases suspected of UTI in Grenada, a tropical Caribbean island nation during the period of 2004 to 2009 were published recently by Hariharan et al.¹⁰ *E. coli* was the predominant species associated with UTI, followed by *Proteus mirabilis* and *Staphylococcus intermedius/Staphylococcus pseudintermedius*. Among six antimicrobials tested against, resistance was least for enrofloxacin, and most for tetracycline. Monitoring changes in antimicrobial efficacy and microbial resistance guides the empirical use of antimicrobials for the treatment of UTI and helps formulate strategic plans to limit drug resistance.⁶ The objectives of this study, therefore, were to examine the overall picture of canine UTI in Grenada for the period of 2010-2016, and to identify drugs which are most likely to be effective for empirical use.

MATERIALS AND METHODS

We conducted a retrospective study of all submissions of urine samples from dogs to the diagnostic bacteriology unit of the St. George's University School of Veterinary Medicine (SGU) for the last 6 years. The laboratory received specimens for routine culture and sensitivity testing from the veterinary clinics in Grenada, including the SGU Small Animal Hospital.

Information on sex, and age of each dog, and treatment administered, if any, and the method of urine collection were collected from the case histories. Urine samples were stored at 5 °C and quantitatively cultured on the same day, and results interpreted as recommended by Osborne.¹ Inoculations were done routinely on blood agar (Columbia agar with 5% sheep blood, Remel Inc., Lennexa, KS, USA) and MacConkey agar (Remel Inc., Lennexa, KS, USA), using a calibrated inoculation loop that delivers 1 µl as per the standard method.¹ After incubation at 37 °C for 18-24 hours, the plates were examined to determine if cultures were pure or mixed, and the colonies were counted. Cut-off values for significant bacteriuria were: ≥1000 colony forming units (CFU)/ ml for samples collected by cystocentesis, ≥10,000 CFU/ml for samples collected by catheterization, and ≥100,000 CFU/ml for voided urine.² Mixed growth of more than 2 colony types was considered as indicative of contamination. Bacterial isolates were identified by the standard phenotypic characteristics as outlined by Quinn et al.¹¹ In case of inconclusive results, API bacterial identification strips (Analytab Products, BioMérieux, Mary-IÉtoile, France, Europe) were

used. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method, as outlined by Quinn et al.¹¹ on Mueller-Hinton (MH) agar. The zone sizes were interpreted as susceptible, intermediate, or resistant, based on the guidelines provided by the Clinical Laboratory Standards Institute (formerly, National Committee for Clinical Laboratory Institute/NCCLS).¹² An *E. coli* strain ATCC 25,922 (American Type Culture Collection), susceptible to all antibiotics, was used as a control.

RESULTS

Of a total of 468 urine samples cultured, 318 were negative for bacterial growth, or had only insignificant growth or mixed growth indicating contamination. Of 150 culture positive samples, indicating urinary tract infection, 109 were from female dogs and 41 from male dogs. The proportion of female dogs with positive bacterial UTI was significantly higher than that of the male dogs ($\chi^2=6.372$, $df=2$, $p=0.006$). The ages of animals varied from 6 weeks to 16 years, and 90% of animals were above one year of age. Eighty seven percent of urine samples had been collected by cystocentesis. Of 23 dogs with a history of antibiotic use, 8 had a course of amoxicillin-clavulanic acid, 2 enrofloxacin, and 1 ciprofloxacin prior to sample submission. The remaining were on doxycycline, amoxicillin, ampicillin-sulbactam, cephalexin, or cefazolin.

Of one hundred and fifty culture-positive samples, none had mixed bacterial infection with 2 species. Table 1 lists the bacteria isolated from the 150 culture-positive samples, and the percentages of various genera and species. Of the 150 isolates, 71.3% were Gram-negative bacteria; the most frequent one was *E. coli* (28.7%), followed by *Proteus mirabilis* (20%), *Klebsiella pneumoniae* (6%) and *Pseudomonas aeruginosa* (4.7%). Among Gram-positive isolates (28.7% of total), the most common species was *Staphylococcus intermedius/Staphylococcus pseudintermedius* (7.3%).

The rates of resistance against 6 antibiotics, that are used to treat UTI in dogs^{3,4,13} are presented in Table 2. Resistance was least (7.8%) to enrofloxacin, when both Gram-negatives and Gram-positives were considered together. However, when Gram-positives alone were taken into account, resistance was least (4.7%) to amoxicillin-clavulanic acid. Resistance was highest to tetracycline, the rate being 61.7% for Gram-negatives, and 50% for Gram-positives.

With regard to *E. coli*, which was the commonest species, none of the isolates were resistant to imipenem (Table 3). Among the other drugs, least resistance was seen to ceftriaxone (4%), and most to cephalothin (71.4%). Of 16 isolates tested against cefotaxime, one was found resistant. Eighteen (42%) of 43 isolates showed resistance to 2 or more classes of drugs. Two isolates showed simultaneous resistance to 6 classes of drugs: An aminoglycoside (gentamicin), beta-lactam drugs (ampicillin, amoxicillin-clavulanic acid, cephalothin), chloramphenicol, quinolones (ciprofloxacin, enrofloxacin), trimethoprim-sulfon-

Table 1: Bacterial Species and the Number of Isolates from 150 Culture-Positive Urine Samples of a Total of 468 Samples Tested from Dogs in Grenada 2010-2016.

Bacterial species	Isolates # (%)	Gram stain id # (%)
<i>Escherichia coli</i>	43 (28.7)	Gram negatives: 107 (71.3) isolates
<i>Proteus mirabilis</i>	30 (20)	
<i>Proteus vulgaris</i>	8 (5.3)	
<i>Klebsiella pneumoniae</i>	9 (6)	
<i>Pseudomonas aeruginosa</i>	7 (4.7)	
<i>Enterobacter cloacae</i>	5 (3.3)	
<i>Enterobacter aerogenes</i>	2 (1.3)	
<i>Pasteurella spp</i>	1 (0.7)	
<i>Serratia marcescens</i>	1 (0.7)	
<i>Serratia rubidaea</i>	1 (0.7)	
<i>Staphylococcus aureus</i>	7 (4.7)	
<i>Staphylococcus intermedius</i>	11 (7.3)	
<i>Staphylococcus spp</i>	10 (6.7)	
<i>Streptococcus canis</i>	1 (0.7)	
<i>Streptococcus uberis</i>	1 (0.7)	
<i>Streptococcus spp</i>	9 (6)	
<i>Aerococcus viridans</i>	3 (2)	
<i>Enterococcus faecalis</i>	1 (0.7)	
Total	150	

Table 2: Antimicrobial Resistance of Bacteria Isolated from UTI in Dogs in Grenada 2010-2016.

Antimicrobials	Resistant Gram-negative isolates/number tested	Resistant Gram-positive isolates/number tested	Total Resistant isolates/ total number tested
Cephalothin	26/43 (60%)	4/17 (23.5%)	30/60 (50%)
Trimethoprim/sulfamethoxazole	13/74 (17.6%)	8/28 (28.6%)	21/102 (20.6%)
Gentamicin	11/96 (11.5%)	3/42 (7.1%)	14/138 (10.2)
Tetracycline	29/47 (61.7%)	8/16 (50%)	37/63 (58.7)
Enrofloxacin	6/103 (5.8%)	5/38 (13.2%)	11/141 (7.8%)
Amoxicillin/clavulanic acid	28/90 (31.1%)	2/43 (4.7%)	30/133 (22.6)

amide, and tetracycline.

All *Proteus mirabilis* isolates tested were susceptible to ceftazidime, and gentamicin. Multiple resistance to 2 or more classes of drugs was seen among 47% of *P. mirabilis*. One of 16 isolates of *E. coli* and 2 of 14 isolates of *P. mirabilis* showed resistance to cefotaxime. All *Pseudomonas aeruginosa* isolates were susceptible to ceftazidime, ciprofloxacin, enrofloxacin, gentamicin, and imipenem, but resistant to tetracyclines. All *Staphylococcus intermedius* isolates were susceptible to amoxicillin-clavulanic acid and enrofloxacin.

DISCUSSION

Of 468 samples, 150 (32.1%) were positive for significant growth in culture, indicative of bacterial UTI. This is somewhat

similar to a recent report from New Zealand where 37.7% of urine samples were positive in culture.¹⁴ All 150 culture-positive samples in the present study had growth of a single bacterial species in high numbers indicative of UTI, and *E. coli* was the most common isolate. These findings are in agreement with the observation that UTIs in dogs are generally caused by a single bacterial species, the predominant being *E. coli*.¹⁵ Several recent studies on UTI in dogs in temperate countries⁶⁻⁹ have shown that *E. coli* is the predominant cause of UTI as seen in the present study and our previous study for the period of 2004-2009 in Grenada.¹⁰ UTI is more common in female dogs, which is in agreement with the results of the previous study in Grenada. In contrast to the results of a study on bacteria from UTI in dogs in Sweden,⁷ drug resistance is high in the dogs in Grenada. Antimicrobial resistance in *E. coli* is of concern in this tropical island, except for imipenem, which is a last-resort drug for multidrug-

Table 3: Antimicrobial Resistance* of *Escherichia Coli* Isolates from UTI in Dogs in Grenada: 2010-2016.

Antimicrobial drugs	Number tested	Resistant	Susceptible	Intermediate
		Number (%)		
Ampicillin (AM)	18	10 (55.5)	5 (27.8)	3 (16.7)
Doxycycline	20	7 (35)	11 (55)	2 (10)
Cephalothin (CF)	21	15 (71.4)	1 (4.8)	5 (23.8)
Trimethoprim/sulfamethoxazole (TMS)	30	6 (20)	22 (73.3)	2 (6.7)
Gentamicin (GE)	36	10 (27.8)	26 (72.2)	0
Tetracycline (TE)	20	10 (50)	10 (50)	0
Chloramphenicol (CL)	15	4 (26.7)	10 (66.7)	1 (6.7)
Enrofloxacin (ENR)	41	3 (7.1)	35 (85.4)	3 (7.3)
Imipenem	39	0	39 (100)	0
Amoxicillin/clavulanic acid (AMC)	32	11 (34.4)	18 (56.3)	3 (9.4)
Ciprofloxacin (CIP)	42	3 (7.1)	39 (92.9)	0
Ceftazidime (CFZ)	38	3 (7.9)	34 (89.5)	1 (2.6)
Cefotaxime (CFT)	16	1 (6.3)	15 (93.8)	0
Cefpodoxime (CFP)	30	5 (16.7)	24 (80)	1 (3.3)
Ceftriaxone (CFR)	25	1 (4)	23 (92)	1 (4)
Cephalexin (CFL)	17	8 (47)	2 (11.8)	7 (41.2)

*Multiple resistance to 6 classes of drugs (AM, AMC, CF, CIP, CL, ENR, GE, TE, TMS) was seen in 2 isolates. One isolate was resistant to cefotaxime with a resistance pattern: AM, AMC, CF, CFL, CFP, CFR, CFT, GE, TE, TMS.

resistant *E. coli*.¹⁶ Over 40% of the *E. coli* isolates in this study showed multiple drug resistance to 2 to 6 classes of drugs, and one isolate was resistant to cefotaxime, which indicates resistance to extended spectrum cephalosporins.⁷ This is of concern in small animal medicine as well as of public health significance when the genes are transferable. Overall, enrofloxacin still remains the most effective drug against urinary pathogens from dogs in this country, more than 92% of all isolates being susceptible. This finding is in agreement with that of a study done in the United States.⁵ Fluoroquinolones are among the few drugs of choice for treatment of bacterial urinary tract infections in dogs, including those due to *Pseudomonas aeruginosa*.¹³ The disk diffusion method underestimates the effectiveness of some drugs, because it is based on expected serum concentrations. Several drugs, including enrofloxacin reach much higher concentrations in the urine.⁴ Although, over half the isolates were resistant to tetracycline, concentrations of doxycycline attained in the urine of dogs can inhibit majority of urinary pathogens.¹⁷ In any case, urine culture remains the “gold standard” for diagnosis of urinary tract infection, and culture and susceptibility testing should particularly be performed in cases that do not respond within 7 days of therapy.³ Prior to obtaining culture and sensitivity results, Gram’s stain may allow identification of the pathogen as Gram-positive or Gram-negative, and this will be helpful in determining initial therapy.¹³

In conclusion, this retrospective study on urinary pathogens from dogs for the period 2010-2016 confirms the results of our previous findings for 2004-2009 that *E. coli* is the most common bacterial species, and resistance to enrofloxacin is minimal. However, emerging multi-drug resistance such as resistance to

cefotaxime, which is generally associated with the production of extended-spectrum beta-lactamases⁷ is of concern. Prudent use of antimicrobial drugs, and continued monitoring of trends in drug resistance are important in controlling bacterial resistance to antibiotics.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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